Proc. Natl. Acad. Sci. USA
Vol. 75, No. 11, pp. 5314–5318, November 1978
Biochemistry

Contacts between *Escherichia coli* RNA polymerase and a *lac* operon promoter

(*Escherichia coli* genetic regulation/polymerase–promoter interactions/DNA cloning/DNA sequencing/RNA nucleotidyltransferase)

LORRAINE JOHNSRUD*

Department of Biology, Harvard University, Cambridge, Massachusetts 02138

Communicated by M. S. Meselson, August 11, 1978

ABSTRACT **The chemical alkylating agent dimethyl sulfate can probe the interaction between *Escherichia coli* RNA polymerase (nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) and the purine bases of a promoter. This agent methylates the N7 position on guanine or the N3 position on adenine; the bound protein can either protect these positions or affect the reactivity to produce an enhanced methylation. The pattern of DNA residues in the lactose promoter protected from, or enhanced to, methylation by a specifically bound polymerase shows that the enzyme covers a region of at least 38 base pairs, stretching upstream from the origin of transcription. These protein–DNA contacts occur predominantly in the major groove of the DNA helix. Furthermore, this pattern of methylation shows that the polymerase unwinds the helix at the origin of transcription. The relationship between polymerase–DNA contacts defined by dimethyl sulfate and known features of promoter structure is discussed. To facilitate these experiments I have constructed a plasmid that permits a unique 5′-end labeling of each strand of a 95-base-pair fragment containing a *lac* operon promoter. This plasmid contains two copies of the *lac* promoter–operator region.**

Escherichia coli RNA polymerase (nucleosidetriphosphate: RNA nucleotidyltransferase, EC 2.7.7.6) initiates transcription at sites on the DNA called promoters. What are the features of these sequences that allow RNA polymerase to recognize them and begin transcription? DNA sequences of prokaryotic promoters are available and reveal two major areas of homology. One is a seven-base region centered ten base pairs upstream from the origin of transcription with canonical sequence T-A-T-R-A-T-G (R, unspecified purine) (1, 2). The second is a region 35 base pairs before the beginning of the messenger RNA identified as the site where mutations and cleavage by restriction endonucleases block promoter utilization (3–8). Mutations in this −35 region can also result in increased promotion (9). However, primary sequence analysis leaves unanswered the question of what specific contacts are formed between RNA polymerase and the DNA of the promoter.

As Gilbert *et al.* (10) showed, one can identify some of the close contacts between a protein and DNA by using the chemical alkylating agent dimethyl sulfate (Me_2SO_4). Me_2SO_4 alkylates the N7 of guanine in the major groove of the DNA helix and the N3 of adenine in the minor groove (11). The glycosidic bond of the methylated purine is labile; heat will remove the base, leaving the sugar, which can be cleaved with alkali (10). If methylation is incomplete, so that only one residue per strand is modified, such a treatment of a DNA fragment bearing a ^{32}P end label at one 5′ end produces a series of fragments corresponding to the site of each guanine and adenine residue in the labeled strand. These products can be resolved by polyacrylamide gel electrophoresis. The comparison of patterns after methylation in the presence and absence of RNA polymerase will define specific purines as sites of close protein–DNA association, because close contacts will block methylation at specific residues.

This approach requires a small restriction fragment, containing the DNA region of interest, that can be labeled uniquely at each 5′ end, independently of the other. Fragments cut out of a longer DNA molecule by two different restriction enzymes are suitable; cleaving with one enzyme followed by labeling both 5′ phosphates and then cleaving with a second enzyme leaves a label on one 5′ end; reversing this procedure labels the other 5′ end. However, the smallest fragment containing the entire *lac* promoter is 95 base pairs long, with sites for cleavage by the restriction endonuclease *Alu* I at both ends (12). Longer DNA fragments carrying the promoter are not convenient because the difficulty of resolving the bases of the promoter on the polyacrylamide gel increases as the distance of the promoter from the 5′-end label increases. Recent advances in gel technology (13) make the use of longer (150 base pair) DNA fragments feasible, but protection and enhancement effects are more easily detected if the region of interest is near the labeled 5′ end.

To obtain uniquely end-labeled promoter DNA easily, I constructed a plasmid (pLJ3) carrying two copies of the 95-base-pair *Alu* I fragment containing the *lac* promoter and operator. The two promoter fragments are fused in specific orientations to an "adapter" DNA fragment. Isolation of plasmid DNA followed by restriction cleavage and end labeling gives quantities of correctly labeled DNA sufficient for extensive experiments with chemical probes. The plasmid has also provided a promoter and ribosome binding site used to maximize expression of a cloned λ repressor gene (14).

Full promotion from the wild-type *lac* promoter occurs only in the presence of the complex of the catabolite-gene activator protein and cyclic AMP (CAP–cAMP). The DNA used to construct pLJ3 contains an "up" promoter mutation that enhances the low level of transcription from the *lac* promoter in the absence of CAP. This mutation, called UV5, increases promotion to 50% of the fully induced wild-type level (15). The UV5 promoter is also a "strong" promoter characterized by a rapid association rate for the polymerase and a long half-time for dissociation (16).

METHODS

Plasmid Construction and Preparation of DNA. *E. coli* K-12 strain 294 (endonuclease I negative, vitamin B_1 requiring, K-12 restriction negative and modification positive; obtained from M. Meselson) was used as a host. The plasmid vectors were

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: Me_2SO_4, dimethyl sulfate; CAP, catabolite-gene activator protein; cAMP, cyclic AMP.

* Present address: Universität Zürich, Institut für Molekularbiologie I, CH-8093 Zürich, Switzerland.

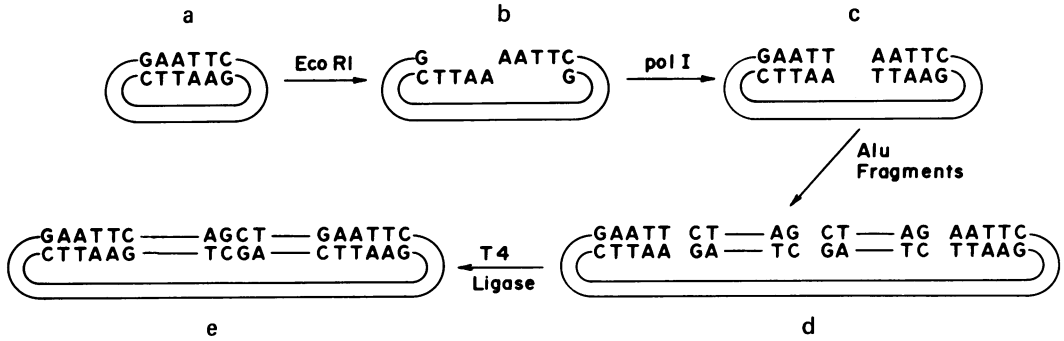

FIG. 1. Cloning random polymers of *lac Alu* fragments. Plasmid $CK\Delta_{11}$ (*a*) is cleaved with *Eco*RI (*b*) and the cohesive ends are filled in with DNA polymerase I (*c*). Plasmid DNA molecules prepared in this way are mixed with *Alu* fragments (*d*) and T4 DNA ligase is added, recreating both *Eco*RI sites and all internal *Alu* sites (*e*). Transformation and screening of recombinant plasmids are described in the text.

$CK\Delta_{11}$ and pMB9. $CK\Delta_{11}$ carries kanamycin resistance determinants; pMB9 confers tetracycline resistance (17). Ligation and transformation were done by the method of Maizels (18), using 0.4 μg of promoter DNA and 0.1 μg of *Eco*RI-digested pMB9 in a 25-μl reaction volume. Transformed cells were allowed to grow overnight in rich broth at 37°C and were plated on plates containing tetracycline (10 μg/ml) and XG, the chromogenic, noninducing substrate 5-chloro-4-bromo-3-indolyl-β-D-galactoside (40 μg/ml) (19). Colonies of transformants synthesizing β-galactosidase constitutively appear blue on such plates. P1 containment conditions were observed.

Plasmid DNA was prepared by the method of Tanaka and Weisblum (20). *Eco*R1 digestions were performed in *Eco*R1 buffer (0.1 M Tris·HCl, pH 7.6/50 mM NaCl/10 mM $MgCl_2$/1 mM dithiothreitol/0.15% Triton X-100). *Hae* III digestions were performed in *Hae* III buffer (50 mM Tris·HCl, pH 7.5/10 mM $MgCl_2$/50 mM NaCl/1 mM dithiothreitol). DNA fragments generated by restriction endonuclease cleavage were purified by electrophoresis on an 8% polyacrylamide TBE (50 mM Tris borate, pH 8.3/1 mM EDTA) gel. End labeling with [γ-^{32}P]ATP and polynucleotide kinase, polyacrylamide gel electrophoresis, and elution of DNA from gels were done as described by Maxam and Gilbert (21).

Methylation Protection. Approximately 1.0 μg of ^{32}P-labeled promoter DNA (20 pmol of labeled 5′ ends) in 0.1 ml of buffer B (50 mM sodium cacodylate, pH 8.0/10 mM $MgCl_2$/50 mM NaCl/1 mM EDTA), was warmed to 37°C before the addition of 180 μg (20-fold molar excess) of *E. coli* RNA polymerase [purified as described by Burgess and Jendrisak (22)]. After 5 min at 37°C, heparin was added to a concentration of 10 μg/ml and the incubation was continued for 15 min at 37°C. A 1-μl sample of 10.7 M Me_2SO_4 was added, and the sample was held at room temperature for 1 min. Reaction mixtures were diluted to 1 ml with buffer B containing 10 mM 2-mercaptoethanol and filtered immediately through a 13-mm Schleicher and Schuell B-6 nitrocellulose filter with gentle suction. Filters were prepared by soaking for 10 min in 1 M KOH, followed by ten rinses with distilled water. DNA was eluted from filters by a 1-hr incubation at 37°C in 0.8 ml of gel elution buffer (21) with 50 μg of tRNA carrier. Samples were then precipitated with 2 ml of ethanol, resuspended in 0.4 ml of 0.3 M sodium acetate, and reprecipitated with 1 ml of ethanol. The pellet was rinsed with 2 ml of cold ethanol and dried under vacuum. At this stage, 30% of the input radioactivity remained. DNA was dissolved in 20 μl of 10 mM sodium phosphate, pH 7.0/1 mM EDTA and heated for 15 min at 90°C; then 2 μl of 1.0 M NaOH

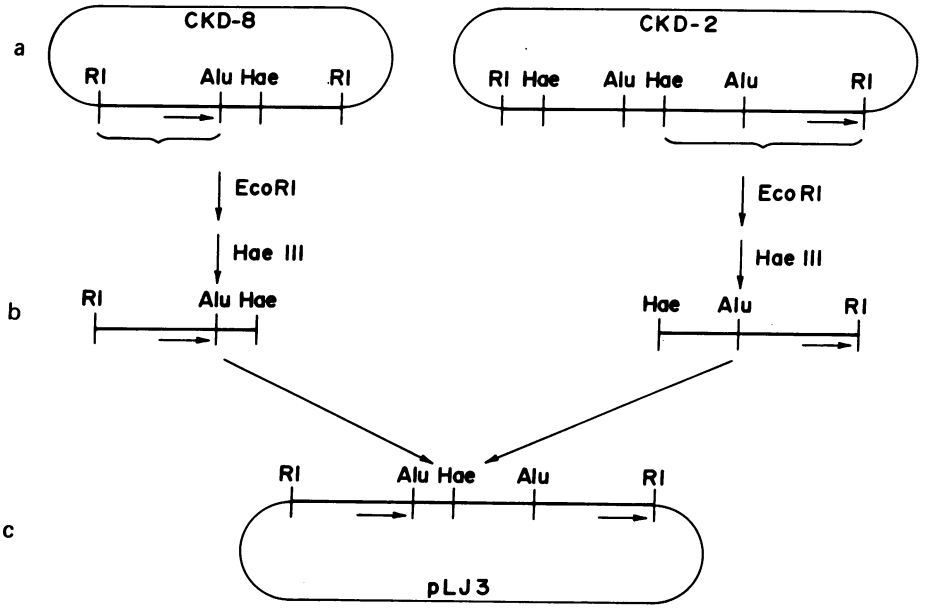

FIG. 2. Construction of plasmid pLJ3. (*a*) Restriction map of inserted *Alu* fragments in plasmids CKD-8 and CKD-2. (*b*) Isolation of two restriction fragments from these inserts that contain the UV5 *lac* promoter in opposite orientations at an *Eco*RI terminus. (*c*) Ligation of these two fragments into *Eco*RI-cut pMB9. Both the *Eco*RI and *Hae* III cleavage sites are recreated. Double-stranded DNA is represented by single lines. Arrows indicate origin and direction of transcription from *Alu* 95 promoter fragments.

was added and the sample was heated in a sealed capillary for 30 min at 90°C. The DNA was added to 20 μl of 10 M urea/0.05% xylene cyanol/0.05% bromphenol blue and electrophoresed for 24 hr at 10 W (constant power) on a 40-cm, 20%, 7 M urea/TBE polyacrylamide gel. 50 counts/sec of radioactivity was loaded in each lane of the gel. A 12-hr exposure on Kodak NoScreen film was used.

RESULTS

Construction of a plasmid with two *lac* promoters

pMB9 is present in 20–30 copies (23). If the plasmid contains a *lac* operator, these 20–30 copies of the operator will bind all the *lac* repressor in the cell. In the absence of repressor, synthesis of β-galactosidase from the chromosomal *lacZ* gene will be constitutive. Thus, transformants containing plasmids bearing *lac* operator DNA will turn blue on indicator plates containing indolyl-galactoside because the constitutive cells contain enough enzyme to cleave this chromogenic substrate readily. *lac* promoter DNA can readily be identified this way because the smallest restriction fragment containing the entire promoter also contains the operator region.

The *lac* operon control region DNA can be isolated by binding sonicated DNA fragments from λ*plac*5 to a filter with *lac* repressor protein (24). Cleaving this purified DNA with *Alu* I produces a 95-base-pair fragment containing the promoter and operator, but at least three other *Alu* I fragments comigrate with this promoter-containing molecule in the polyacrylamide gel used to separate the products of the *Alu* I digestion. Plasmids were constructed containing random polymers of the UV5 *Alu* 95 promoter fragment and the comigrating contaminants. The plasmid vector, $CK\Delta_{11}$, was cleaved with *Eco*RI at the unique *Eco*RI site, and the protruding 5′ ends that resulted were filled in with DNA polymerase I. This DNA was mixed with *Alu* 95 fragments and treated with T4 DNA ligase, resulting in the simultaneous formation of random *Alu* polymers and their flush-ended ligation into the plasmid. DNA concentrations of the cleaved plasmid and the *Alu* fragments were manipulated to maximize insertion of multiple fragments into the vector (25). The circular molecules resulting from this ligation were used to transform strain MM294. β-Galactosidase-constitutive kanamycin-resistant colonies appeared after transformation; twelve separate clones were designed CKD-1 through CKD-12.

Flush-ended ligation of the repaired *Eco*RI site with the *Alu* fragments recreates two *Eco*RI cleavage sites [Fig. 1 (26)]; thus, the *Alu* polymers can be excised with *Eco*RI. I isolated plasmid DNA from CKD-5 through CKD-12, cleaved it with *Eco*RI, and labeled the 5′ ends with [γ-^{32}P]ATP and polynucleotide kinase. The smaller labeled *Eco*R1 fragments were polymers of two or more *Alu* fragments. I isolated these polymers and digested them with *Hae* III, which cut in the contaminating fragments but not in the promoter DNA fragment.

I reasoned that in this collection of *Eco*RI/*Hae* III fragments from the different plasmids those with lengths between 100 and 200 base pairs could contain a *lac* promoter fragment labeled at an *Eco*RI terminus attached to other DNA from an *Alu* to a *Hae* cut. By partial sequencing (21) of different *Eco*RI/*Hae* III fragments I found two of different lengths that carried the promoter in different orientations (Fig. 2). The isolation of these fragments would solve the problem of introducing a separate label on each end of the promoter; however, the useful fragments were contained in two different plasmid clones. To simplify the preparation of DNA for methylation experiments I combined the two fragments shown in Fig. 2*b* in order to make pLJ3. The two DNA molecules were mixed with *Eco*RI-digested pMB9, ATP, and T4 DNA ligase. This resulted in annealing and sealing of the *Eco*RI cohesive ends as well as flush-ended ligation of the internal *Hae* III site (Fig. 2*c*). Thus, DNA for methylation experiments is prepared by cleaving pLJ3 DNA with *Eco*RI, eluting the resulting fragment from a gel, end labeling, cleaving with *Hae* III, and isolating the uniquely end-labeled fragments from a second gel.

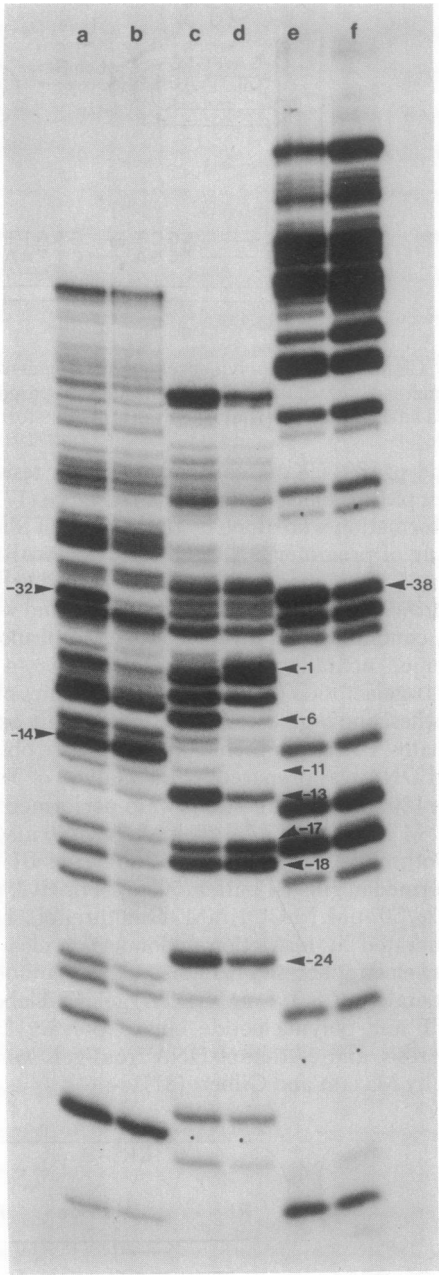

FIG. 3. Autoradiograph showing effects of bound polymerase on methylation. DNA is separately labeled on the bottom (lanes a and b) and top (lanes c–f) strands. "Bottom" and "top" are as defined in Fig. 4. Lanes e and f were loaded after the bromphenol blue in lanes a–d had reached the bottom of the gel. Arrows point to residues at which reproducible effects occur, and numbers correspond to the numbering of the DNA sequence in Fig. 4. Lanes a, c, and f were methylated in the absence of polymerase, lanes b, d, and e, in the presence of polymerase.

Methylation of RNA polymerase DNA complexes

I found that specific protection by the polymerase could only be observed by eliminating all nonspecific polymerase-DNA complexes. Heparin competes with DNA for binding to polymerase (27). The complex at the *lac* promoter is reasonably

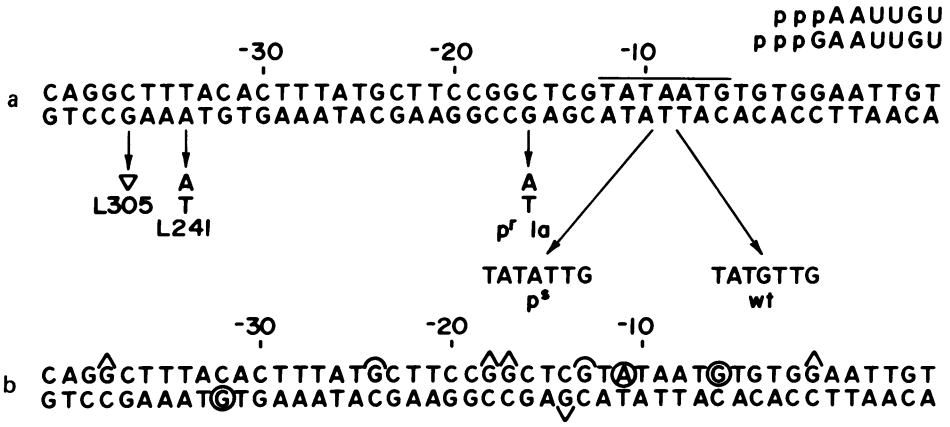

FIG. 4. Results of methylation protection experiments with RNA polymerase and the *lac* UV5 promoter. (*a*) The *lac* promoter region. The Pribnow box is indicated by a line. Promoter mutations are described in the text. Wild-type (wt) sequence is from Dickson *et al.* (6). (*b*) Residues at which methylation is affected by bound polymerase. Circled bases are blocked to methylation; half circles indicate partial blockage; carets above bases show points of enhanced methylation.

resistant to heparin. Nonspecific complexes, in which the polymerase is randomly bound to nonpromoter DNA, are less stable (28). The specific complexes remaining after the heparin chase are separated from unbound labeled DNA by passing the solution through a cellulose nitrate filter (29). RNA polymerase is retained on the filter; free DNA passes through. Only DNA molecules complexed to polymerase remain on the filter. It is this labeled DNA that is eluted from the filter, cleaved with alkali, and displayed on a gel. The requirement for heparin and filter binding could be specific for this promoter. Alternatively, the use of more extensive purified or σ-saturated polymerase might make these steps unnecessary.

The effects shown below occur reproducibly in every repetition of the protection experiment. Minor irreproducible variations among experiments do occur, however, even using the same RNA polymerase and DNA preparations. Densitometer scans were used to confirm the protections and enhancements. "Complete" blockage of methylation results in a reduction of at least 80% in the intensity of the band on the autoradiogram. Enhancements increase the band intensity 50% or more. It should be noted that variations of the protection experiment are possible, including methylation of DNA prior to polymerase binding.

Fig. 3 shows an autoradiograph of the methylation experiment. The relative intensities of the bands (G darker than A) reflects the severalfold greater rate of methylation at N7 of guanine over N3 of adenine (11). The purines are easily correlated with the known sequence of the UV5 promoter (J. Gralla, personal communication). Fig. 4 shows the positions in the sequence affected in this experiment. Methylation of guanine residues at −6 in the top strand and −32 in the bottom strand is blocked by polymerase. At −13 and −24 in the top strand the guanines are partially blocked. These are most easily interpreted as regions where the protein is so closely associated with the DNA in the major groove that the small Me_2SO_4 molecule cannot reach its N7 target. The only such contact seen in the minor groove is the adenine residue in the top strand at position −11.

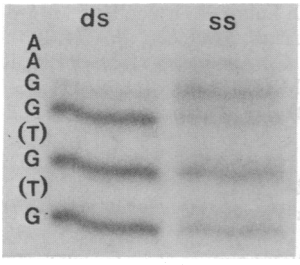

FIG. 5. G-G-A sequence effect at −1 in the *lac* UV5 promoter. Methylation of the second G in the G-G-A sequence is suppressed when the DNA molecle is double stranded (ds). This effect is not seen when single-stranded DNA is methylated (ss). Positions of Ts in the sequence are indicated in parentheses. Single-stranded DNA was methylated without $MgCl_2$ but in the presence of EDTA or 90% formamide in order to demonstrate this methylation effect. Presumably, inverted repeat sequences on the single-stranded promoter DNA lead to the formation of stem and loop structures. Denaturing conditions destroy such hairpin loops and allow equal methylation to occur at both Gs in the G-G-A sequence.

Guanines at −1, −17, −18, and −38 in the top strand and −14 in the bottom strand are methylated more strongly in the presence of polymerase. This enhancement is currently unexplained. Although it could be due to a slight change in the electronic structure of the DNA, the most likely explanation, considering the pattern of enhancement, is that these bases occur at an edge of a protein–DNA contact region and may represent a "hydrophobic pocket" that binds Me_2SO_4 sulfate slightly and increases its effective concentration. However, at least one enhancement, the G at −1, can be explained as the removal of a sequence-dependent effect. In general, on double-stranded DNA, the reactivity of the middle guanine in a G-G-A sequence is suppressed, a sequence-specific effect that does not occur on single-stranded DNA (19). Specifically, this is true for the G-G-A sequence on the top strand near the origin of transcription in the *lac* promoter. I compared the methylation pattern of double-stranded DNA with that obtained if this region is denatured and is single stranded. Fig. 5 shows that the methylation of the G at −1 is suppressed on a double-stranded promoter fragment, and thus apparently enhanced when the strands are separated. RNA polymerase is known to unwind the DNA helix 1–1½ turns (30, 31). I conclude that the polymerase, unwinding the DNA helix, creates a localized single-stranded region that is responsible for the enhanced reactivity of the guanine at −1.

DISCUSSION

Methylation-defined contacts in the *lac* UV5 promoter appear predominantly at guanines, in the major groove of the DNA helix. Yet Melinkova *et al.* (30) detect only a small effect of bound RNA polymerase on production of 7-methylguanine by using phage T7 or calf thymus DNA. In these experiments polymerase is bound, followed by methylation and DNAse digestion. The production of 7-methylguanine in the digested

and polymerase-protected, intact DNA is compared. Methylation of a large number of nonspecific polymerase binding sites gives an average effect; results for an individual site could vary from this mean. In addition, the summation of protections and enhancements could result in no net effect. In the UV5 promoter four Gs are blocked to methylation while five are enhanced. The overall effect would be a small increase in 7-methylguanine production in the presence of polymerase.

The pattern of contacts defined by Me_2SO_4 corresponds to several features of the primary DNA sequence of promoters. Me_2SO_4 contacts occur in the two regions identified as important to polymerase site selection and binding—the Pribnow box at −6 to −12 and the recognition region at −35. The only minor groove contact seen between the polymerase and the DNA is the adenine at −11 in the Pribnow box. This adenine is strongly conserved in all promoter sequences (16). In addition, it is part of a two-base overlap between two Pribnow box sequences preceding the separate CAP-dependent and CAP-independent transcription initiation sites in the galactose operon (32).

The other region of the promoter important in polymerase recognition is centered at −35. DMS identifies contacts at −32 and −38. However, the Me_2SO_4 contacts do not occur at sites of sequenced promoter mutations, either "down" mutations (L305 and L241) or "up" mutations (p^s, p^r 1a, and UV5) in or near the Pribnow box (33).

These methylation-defined contacts are spread over a region stretching from the origin of transcription to a point 38 base pairs upstream. Thus, this experiment argues that the polymerase binds simultaneously to the recognition region at −35, the Pribnow box region at −10, and the initiation region around −1. Small-angle x-ray measurements in solution suggest that the holoenzyme monomer has an elongated dimension of 150 Å, enough to cover 30–40 base pairs (34). The DNA must wind partially about the polymerase, if that molecule is to contact 40 bases upstream from the origin of transcription and protect another 20 bases downstream from DNAse (refs. 1, 2, 35, 36; J. Gralla, personal communication). This pattern of contacts could not result from two or more populations of DNA molecules with polymerases bound at different sites because this would make it impossible to completely block methylation at any residue. The presence of polymerase at −35 opens the possibility of physical contact between the polymerase and the CAP–cAMP complex bound at the −60 CAP site (37).

I thank Robert Simpson and John Majors for RNA polymerase, Allan Maxam for kinase, and Nancy Maizels for ligase. I also thank A. Maxam for advice and discussion and W. Gilbert for guidance and help with the manuscript. I was supported by a National Science Foundation Predoctoral Fellowship and Grant GMS-09541 from the National Institutes of Health to W. Gilbert.

1. Pribnow, D. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 784–788.
2. Schaller, H., Gray, C. & Hermann, K. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 737–741.
3. Allet, B., Roberts, R. J., Gesteland, R. F. & Solem, R. (1974) *Nature (London)* **249,** 217–220.
4. Hopkins, J. (1974) *J. Mol. Biol.* **87,** 715–724.
5. Maurer, R., Maniatis, T. & Ptashne, M. (1974) *Nature (London)* **249,** 221–223.
6. Dickson, R. C., Abelson, J., Barnes, W. M. & Reznikoff, W. S. (1975) *Science* **187,** 27–35.
7. Meyer, B., Kleid, D. & Ptashne, M. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 4785–4789.
8. Kleid, D., Humayan, Z., Jeffrey, A. & Ptashne, M. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 293–297.
9. Calos, M. (1978) *Nature (London)* **274,** 762–765.
10. Gilbert, W., Maxam, A. & Mirzabekov, A. (1976) in *Control of Ribosome Synthesis* Alfred Benzon Symposium IX, eds. Kjeldgaard, N. O. & Maaløe, O. (Academic, New York), pp. 139–148.
11. Lawley, P. D. & Brooks, P. (1963) *Biochem. J.* **89,** 127–138.
12. Gilbert, W., Gralla, J., Majors, J. & Maxam, A. (1974) in *Protein-Ligand Interactions*, eds. Sund, H. & Blauer, G. (de Gruyter, Berlin).
13. Sanger, F. & Coulson, A. R. (1978) *FEBS Lett.* **87,** 107–110.
14. Backman, K. & Ptashne, M. (1978) *Cell* **13,** 65–71.
15. Arditti, R. R., Scaife, J. & Beckwith, J. (1968) *J. Mol. Biol.* **38,** 421–426.
16. Majors, J. (1977) Dissertation (Harvard University, Cambridge, MA).
17. Rodriguez, R. L., Bolivar, F., Goodman, H. M., Boyer, H. W. & Betlach, M. (1976) in *Proceedings of the ICN-UCLA Symposium on Molecular and Cellular Biology*, eds. Nierlich, D. P., Rutter, W. S. & Fox, C. F. (Academic, New York), pp. 23–27.
18. Maizels, N. (1976) *Cell* **9,** 431–438.
19. Miller, J. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
20. Tanaka, T. & Weisblum, B. (1975) *J. Bacteriol.* **121,** 354–362.
21. Maxam, A. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 560–564.
22. Burgess, R. R. & Jendrisak, J. J. (1975) *Biochemistry* **14,** 4634–4638.
23. Clewell, D. B. & Helinski, D. R. (1972) *J. Bacteriol.* **110,** 1135–1146.
24. Gilbert, W. & Maxam, A. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 3581–3584.
25. Dugaiczyk, A., Boyer, H. W. & Goodman, H. M. (1975) *J. Mol. Biol.* **96,** 171–184.
26. Backman, K., Ptashne, M. & Gilbert, W. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 4174–4178.
27. Zillig, W., Zechel, K., Rabussay, D., Schachner, M., Sethi, V. S., Palm, P., Heil, A. & Seifert, W. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35,** 47–58.
28. Chamberlin, M. J. (1976) in *RNA Polymerase*, eds. Losick, R. & Chamberlin, M. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
29. Hinkle, D. C. & Chamberlin, M. J. (1972) *J. Mol. Biol.* **70,** 157–185.
30. Melinkova, A., Beabealashvilli, R. & Mirzabekov, A. D. (1978) *Eur. J. Biochem.* **84,** 301–309.
31. Wang, J. C., Jacobsen, J. H. & Saucier, J. M. (1977) *Nucleic Acids Res.* **4,** 1225–1241.
32. Musso, R., Di Lauro, R., Rosenberg, M. & de Crombrugghe, B. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 106–110.
33. Dickson, R. C., Abelson, J., Johnson, P., Rezinikoff, W. S. & Barnes, W. M. (1977) *J. Mol. Biol.* **111,** 65–75.
34. Pilz, I., Kratky, O. & Rabussay, D. (1972) *Eur. J. Biochem.* **28,** 205–220.
35. Heyden, B., Nusslein, C. & Schaller, H. (1972) *Nature (London) New Biol.* **240,** 9–12.
36. Walz, A. & Pirrotta, V. (1975) *Nature (London)* **254,** 118–121.
37. Majors, J. (1975) *Nature (London)* **256,** 672–674.